Reviewer’s report

Title: GLUT1 gene is a potential hypoxic and prognostic marker in colorectal cancer patients

Version: 1 Date: 20 September 2008

Reviewer: Lothar Fecker

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Date: 20.09.2008

Title:

Glut1 gene is a potential hypoxic and prognostic marker in colorectal cancer patients” by Fu-Yen Chung Ming-Yii Huang, Ching-Sheng Yeh, Tian-Lu Cheng, Li-

Chen Yen, Jaw-Yuan Wang, Shiu-Ru Lin

In the above mentioned manuscript Fu-Yen Chung et al. report their data on expression of hypoxia-related and glycolysis-related genes in colorectal cancer tissues and colorectal adenocarcinoma cell lines under normoxic and hypoxic conditions. As hypoxia is a characteristic feature of aggressive tumors, the authors investigated whether typical marker genes for hypoxia can be identified in colorectal cancer patients, which may be applicable for diagnosis, prognosis and monitoring of recurrence after treatment.

They first show that lactate concentration significantly is increased in the cell lines under hypoxic conditions, and in parallel they demonstrate increased expression of the transcription factor HIF-1# and of the GLUT1 (a glucose transporter protein) mRNA by RT-PCR. Then they investigated mRNA expression of seven glycolysis and nine hypoxia–related genes in 10 CRC tissue specimens as well as in colorectal tissues of healthy volunteers using oligonucleotide-based membrane arrays. GLUT1 mRNA, HIF-1# and HIF-2# mRNA turned out to be among the transcripts with highest induction factors in tumor tissues compared to normal tissues. Finally the authors show by mRNA membrane array that in peripheral blood specimens of 100 CRC patients the GLUT1 gene is the only gene among five additional genes investigated for which there was a significant correlation between mRNA expression and progression.

1. The questions posed by the authors are well defined.

2. The methods overall are appropriate and well described (for comments see below).

3. The data are presented in a convincing way, however statistic evaluation should be revised by a statistician.

4. Also reporting and data deposition are according the relevant standard for
reporting and data deposition.

5. The discussion and conclusions should be extended:
   see below

6. Also the limitation of the work should be mentioned in the discussion
   See below

7. The authors acknowledge the work upon which they are building.

8. The title and abstract accurately convey what has been found.

9. The writing is acceptable.

In addition I have the following suggestions for improvement of the manuscript:

-Major Compulsory Revisions (which the author must respond to before a
decision on publication can be reached)

1. Page 13, Chapter: HIF-1 and GLUT1 mRNA expression in SW480 and---
   Although the PCR products have been quantified in relation of the housekeeping
   -actin signal the RT-PCR method is only a semiquantitative method.
   Furthermore it would be a gain for the publication if the authors show that -actin
   expression, which has been used as an internal standard, is shown identical
   under normoxic and hypoxic conditions in the cell lines analysed.

2. Figure 6: In the title of figure 6 it is mentioned that relative gene expression
   level is shown for case 6. I think the authors mean from sample CRC6. But later
   it is stated that the overexpression of Glut1, Hif-1### and Hif-2#### are presented in
   all ten human colorectal cancer tissue samples. This is a contradiction! If for
   these 3 proteins mean values of all 10 samples are shown in the diagram the
   values should be different and the standard deviation would also be higher.
   Please check the diagram and the figure legend carefully and clearly describe
   what is shown in the figure.

-Minor Essential Revisions (such as missing labels on figures, or the wrong use
of a term, which the author can be trusted to correct)

1. Page 6, Introduction
   Please check Ref. 18

2. Page 7, Chapter: Lactate concentration measurements
   To which glucose analogs the authors refer to?

3. Page 8, Chapter: Reverse transcription-polymerase chain reaction (RT-PCR)
   Lane 2: Two microliters of cDNA were used for each reaction.
   Where does the cDNA come from?
   Please describe cDNA synthesis and the amount of RNA used for cDNA
synthesis!

4. Page 10, Chapter, Total RNA extraction and cDNA synthesis
Sentence: First strand cDNA was synthesized from total RNA…
Please mention amount of RNA used for cDNA synthesis!

5. Page 11, Chapter: Hypoxia and glycolysis-associated genes …
Abbreviation TB should be explained here!

6. Page 11, Chapter: Preparation of digoxigenin-labelled …
Have the authors used (DIG)-labelled dUTP instead of (DIG)-labelled UTP?

7. Page 12, Chapter: Preparation of digoxigenin-labelled …
Please write bacterial gene instead of bacteria gene!

8. Page 12, same Chapter as 4.
Lane 6: Sixteen target genes…
I think this part should be integrated into the previous chapter in which membrane array preparation is described.

10. The discussion and conclusions should be extended:
Questions to be discussed:
Is GLUT1 expression also elevated in other tumor entities?
Are there any contradictory reports on expression of GLUT1 under hypoxic conditions?

11. Also the limitation of the work should be mentioned in the discussion
For example: is GLUT1 expression level in the blood a reliable prognostic marker for CRC patients and how could this be confirmed and investigated further?

Discretionary Revisions (which are recommendations for improvement but which the author can choose to ignore):
None

**Level of interest**: An article of importance in its field

**Quality of written English**: Acceptable

**Statistical review**: Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests**:
I declare that I have no competing interests